ELECTROPHORETIC PREPARATION OF THE TWO RAT α-FETOPROTEIN VARIANTS

J. P. KERCKAERT, B. BAYARD, S. QUIEF and G. BISERTE

Institut de Recherches sur le Cancer de Lille (Institut Jules Driessens) et Unité n° 124 de l'INSERM, BP n° 3567, 59020 - Lille Cedex, France

Received 10 March 1975

1. Introduction

Alphafetoprotein (AFP) has already been purified from various sources by several convenient methods [1–4]. Rat AFP has been found heterogeneous on electrophoresis in low porosity polyacrylamide gels [1,4–6]. However, the nature of the microheterogeneity remains unclear due to the difficulty to purify two AFP variants in sufficient amounts.

Using a simple preparative procedure of polyacrylamide gel electrophoresis we were able to isolate the two Rat AFP and to compare some of their respective biochemical and immunological properties.

2. Materials and methods

2.1. Source of AFP

Rat AFP was obtained from amniotic fluid collected between the 14th and the 18th day of gestation. The amniotic fluid was clarified by centrifugation and stored at −20°C.

2.2. Preparative electrophoresis

Preparative electrophoresis was performed on polyacrylamide slab gel (0.5 cm thick, 15 cm high; 20 cm wide) in the apparatus described by Kaltschmidt and Wittmann [7].

A discontinuous gel system was prepared according to Davis [8] using a 12% acrylamide concentration in the lower gel and a 0.125 M Tris–HCl, pH 8.5, buffer. After layering the sample (2 ml amniotic fluid per slab), the gels were run at 120 V for 15 hr. Two lateral guide strips (0.5 cm wide) were cut out from the slab and stained for 10 min with 1% Amido Black 10 B in acetic acid–ethanol–water (5:20:75). After electrical destaining, the areas corresponding of the two AFP variants (AFP_A and AFP_B) were cut off from the unstained gel and ground in 0.15 M NaCl.

The mixture was then stirred for 1 hr and centrifuged; this treatment was repeated three times. The pooled extracts were passed through a Millipore filter (0.45 μm pore size) and concentrated by ultrafiltration on a PM 30 Diaflo membrane.

AFP was further purified by gel chromatography on Sephadex G-100 in 0.15 M NaCl in order to remove some impurities coming from the polyacrylamide gel.

2.3. Analytical gel electrophoresis

Analytical gel electrophoresis was performed with the same gel system as above on scale-down slabs (0.2 × 10 × 10 cm). SDS (sodium dodecylsulfate) gel electrophoresis was carried out according to the Neville's method [9].

2.4. Antisera

Monospecific antisera against AFP_A and AFP_B were prepared by immunizing a rabbit with 4 mg of purified AFP_A or AFP_B. Each injection contained 1 mg of antigen dissolved in 0.5 ml of 0.15 M NaCl and emulsified with 0.5 ml of Freund's complete adjuvant (Difco).

2.5. Neuraminidase treatment

Enzymatic digestions of AFP_A and AFP_B were realized with commercial neuraminidase (EC 3.2.1.18) isolated from *Clostridium perfringens* [10]. Controls were incubated without the enzyme.

2.6. Chemical analyses

Amino acids analyses were performed with a

North-Holland Publishing Company – Amsterdam

Beckman amino acid analyzer (Multichrom) on samples hydrolyzed under vacuum in 5.6 N HCl at 110°C for 24 hr. Molar ratios of monosaccharides were obtained by classical quantitative gas-liquid chromatography [11].

3. Results

As shown in fig.1, the two AFP and albumin from rat amniotic fluid are well separated by preparative electrophoresis on polyacrylamide gel slab. The recovery of the proteins from the gel is about 40%. However, the fast variant, AFP_B, is slightly contaminated by albumin. This contaminant is removed by re-running AFP_B in preparative electrophoresis. Under analytical electrophoresis (fig.2) AFP_A and AFP_B appear to be obtained in a pure form; presence of α_1-acid glycoprotein, albumin or transferrin contamination is not detectable. After neuraminidase treatment the two variants have a decreased electrophoretic mobility but are still well separated (fig.2). When analyzed by the SDS procedure, the two AFP preparations give the same single band (fig.3) corresponding to an approximate mol. wt of 70 000.

Immunodiffusion experiments carried out with purified AFP_A and AFP_B against rabbit antisera anti-

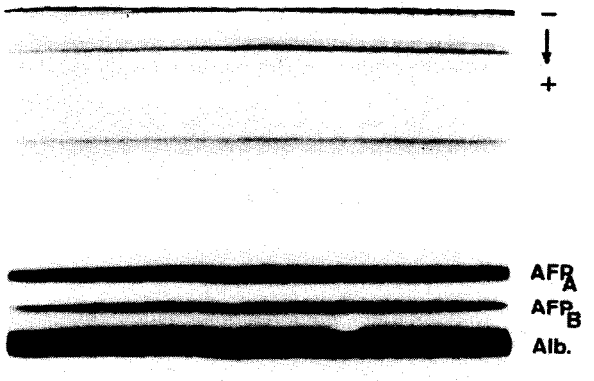

Fig.1. Preparative slab gel electrophoresis of rat amniotic fluid. The slab was entirely stained in order to show the good separation of the variants AFP_A and AFP_B and the reliability of the method.

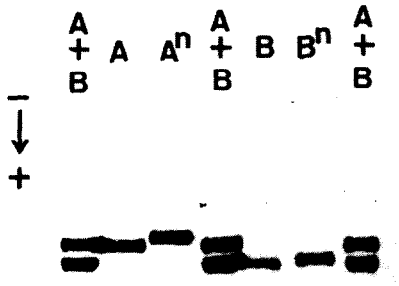

Fig.2. Analytical slab gel electrophoresis of the two purified AFP variants (A and B) and effect of neuraminidase treatment (A^n, B^n) on electrophoretic mobilities.

Fig.3. SDS electrophoresis of purified rat AFP variants. 100 μg samples of each protein were applied. The gels were stained with Coomassie Blue.

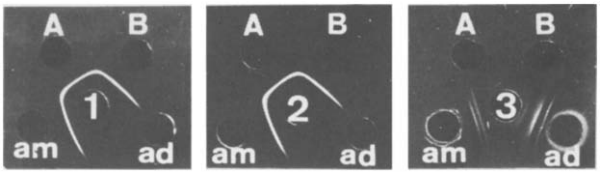

Fig.4. Immunodiffusion patterns of AFP_A (A), AFP_B (B), amniotic fluid (am) and adult rat serum (ad) against rabbit antisera (central well): 1: antiAFP_A; 2: antiAFP_B; 3: Anti adult rat serum.

AFP_A or antiAFP_B and anti adult rat serum (fig.4) confirm the antigenic identity of the two AFP [4–6] and further indicate they are free of protein contaminant.

Amino acid and carbohydrate compositions of AFP_A and AFP_B are given in table 1. From these results no significant variations to which the difference of the electrophoretic mobilities could be related, appears between the two variants.

Table 1
Carbohydrate and amino acid compositions of the two rat AFP

	α-Fetoprotein	
	AFP_A	AFP_B
Carbohydrates (mol/mol of AFP)		
Galactose	6.2	5.8
Mannose	6.0	6.0
N-acetyl glucosamine	7.6	7.8
N-acetyl neuraminic acid	6.2	5.8
Amino acids (per cent residues)		
Aspartic acid	8.7	8.6
Threonine	5.0	5.2
Serine	8.6	8.0
Glutamic acid	15.5	15.3
Proline	4.2	4.3
Glycine	6.4	7.9
Alanine	8.2	8.5
Cysteine	3.9	4.0
Valine	4.2	4.2
Methionine	2.0	1.7
Isoleucine	4.5	4.0
Leucine	9.3	8.9
Tyrosine	2.4	2.4
Phenylalanine	4.3	4.3
Lysine	6.9	7.0
Histidine	2.9	2.8
Arginine	3.4	3.4

4. Discussion

Separation and purification of the two molecular forms of rat AFP can only be achieved by electrophoretic procedure. Such a preparative method has already been described [1] but was not used to separate AFP variants. Indeed, for this purpose, electrophoresis must be performed on low porosity gels on which AFP_A, AFP_B and albumin migrate in very distinct zones. Electrofocusing has also been shown an effective tool to characterize the microheterogeneity of human AFP [3], but its use on a preparative scale is rather tedious and expensive.

The procedure reported here allowed us to obtain the two rat AFP in a good yield. Similarity in carbohydrate and amino acid compositions suggests that the microheterogeneity may be due to conformational changes or in slight net charge differences. Thus, the precise nature and significance of AFP heterogeneity must await further structural and functional studies using the purified variants.

Acknowledgements

This work was supported by grants from the Centre National de la Recherche Scientifique and the Institut National de la Santé et de la Recherche Médicale.

References

[1] Gusev, A. I. and Yazova, A. K. (1970) Biokhimiya 35, 172–181.
[2] Nishi, S. and Hirai, H. (1972) Biochim. Biophys. Acta 278, 293–298.
[3] Alpert, E., Drysdale, J. W. and Isselbacher, K. J. (1973) Ann. N. Y. Acad. Sci. 209, 387–396.
[4] Cittanova, N., Grigorova, A. M., Benassayag, C., Nunez, E. and Jayle, M. F. (1974) FEBS Lett. 41, 21–24.
[5] Aussel, C., Uriel, J. and Mercier-Bodard, C. (1973) Biochimie 55, 1431–1437.
[6] Watabe, H. (1974) Int. J. Cancer 13, 377–388.
[7] Kaltschmidt, E. and Wittmann, H. G. (1970) Anal. Biochem. 36, 401–412.
[8] Davis, B. J. (1964) Ann. N. Y. Acad. Sci. 121, 404–427.
[9] Neville, D. M., Jr. (1971) J. Biol. Chem. 246, 6328–6334.
[10] Cassidy, J. T., Jourdian, G. T. and Roseman, S. C. (1965) J. Biol. Chem. 240, 3501–3506.
[11] Zanetta, J. P., Breckenridge, W. C. and Vincendon, G. (1972) J. Chromatogr. 69, 291–304.